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Short communication

Chondroitin sulfate and other sulfate containing chondroprotective agents may exhibit their effects by overcoming a deficiency of sulfur amino acids

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Dietary supplements derived from cartilage and medications containing sulfated amino sugars, are used with increasing frequency by individuals affected with osteoarthritis (OA). Our lack of knowledge regarding their mode of action is further complicated by the fact that many of these compounds have to be degraded by the intestinal flora for absorption. The metabolic studies performed in this work suggest that the sulfate moiety present in these molecules may be overcoming a dietary deficiency of sulfur amino acids (SAA; cysteine and methionine), our primary sources of sulfate. The compounds tested are rapidly converted into free sulfate before or after absorption, depending on their chemical nature, and excreted quantitatively when the intake of protein seems to exceed the dietary requirement for the SAA. At lower levels of protein intake, sulfur is retained and excretion considerably reduced.

Cartilage is a unique tissue that after acquiring its full complement of cells during early development expands in size by deposition of collagen type II, and tissue specific glycosaminoglycans (GAG) that require a source of inorganic sulfate for their synthesis. After maturity, the turnover of these molecules is very limited. Classical studies by Mankin and Maroundas estimated a $T_{1/2}$ of 3.5 years for some proteoglycan fractions and 25 years for most others[1–3]. Sub-optimal amounts of SAA, our major sources of inorganic sulfate used for the de novo synthesis of GAG, may not cause any significant problem to healthy cartilage, but when OA begins to progress could lead to serious consequences. During the onset of disease GAG turnover is greatly enhanced, as evidenced by the rapid loss of metachromasia. It is at this time, that it becomes critical to be able to replace the matrix components, particularly the GAG which seems to be most vulnerable to degradation. In the context of this study it seemed to us rather coincidental that essentially all the dietary supplements as well as many medications claimed or shown to alleviate symptoms of OA contain large amounts of sulfur (chondroitin sulfate, glucosamine sulfate, SAMe (S-adenosyl methionine), MSM (methyl-sulfonyl-methane) etc.).

The requirements for essential amino acids established over 50 years ago in young college students, takes only into account the amounts needed to restore nitrogen balance[4,5], yet it should be readily apparent that the SAA are in a class of their own. In addition to being building blocks for proteins, they are the primary source of sulfur used in the synthesis of many key metabolic intermediates as well as GAG, main components of the extracellular matrix.

These facts led us to believe that dietary sulfur may play an important role in the progression of OA and that the recommended dietary allowance (RDA) for SAA methionine and cysteine may underestimate the body needs for these mutually complementary essential nutrients, particularly during periods of increased synthesis, in the aged, malnourished and individuals with joint pains who are using acetaminophen, since 40% of this medication is excreted in the urine conjugated with sulfate.

With this in mind we decided to investigate the relationship between dietary levels of protein and urinary excretion of sulfate during the administration of a single supplement of 10 mmoles of L-methionine, to try and fill a gap in our limited knowledge on sulfur balance. This study was followed by another one where dietary supplements were used in equivalent molar amounts. Subjects were normal human volunteers, aged 41–70, who were instructed to record their dietary intake and to carefully save aliquots of their urinary output. Sulfate (free and esterified) was measured by a modification of the nephelometric method of Berglund and Sorbo[6] and levels of SAA intake evaluated using a Nutritional Analysis Software (ESHA Research, version 7.6). L-Methionine (Solgar, Leonia NJ), chondroitin sulfate (Sigma) and glucosamine sulfate (Metagenics, San Clemente, CA) were purchased. By chemical analysis it was found that 52% of the glucosamine was sulfated (11 tablets of 500 mg each supplied 10 mmoles of sulfate). Patients were adapted to a particular level of dietary protein by starting them on their diets at least 24 h in advance of

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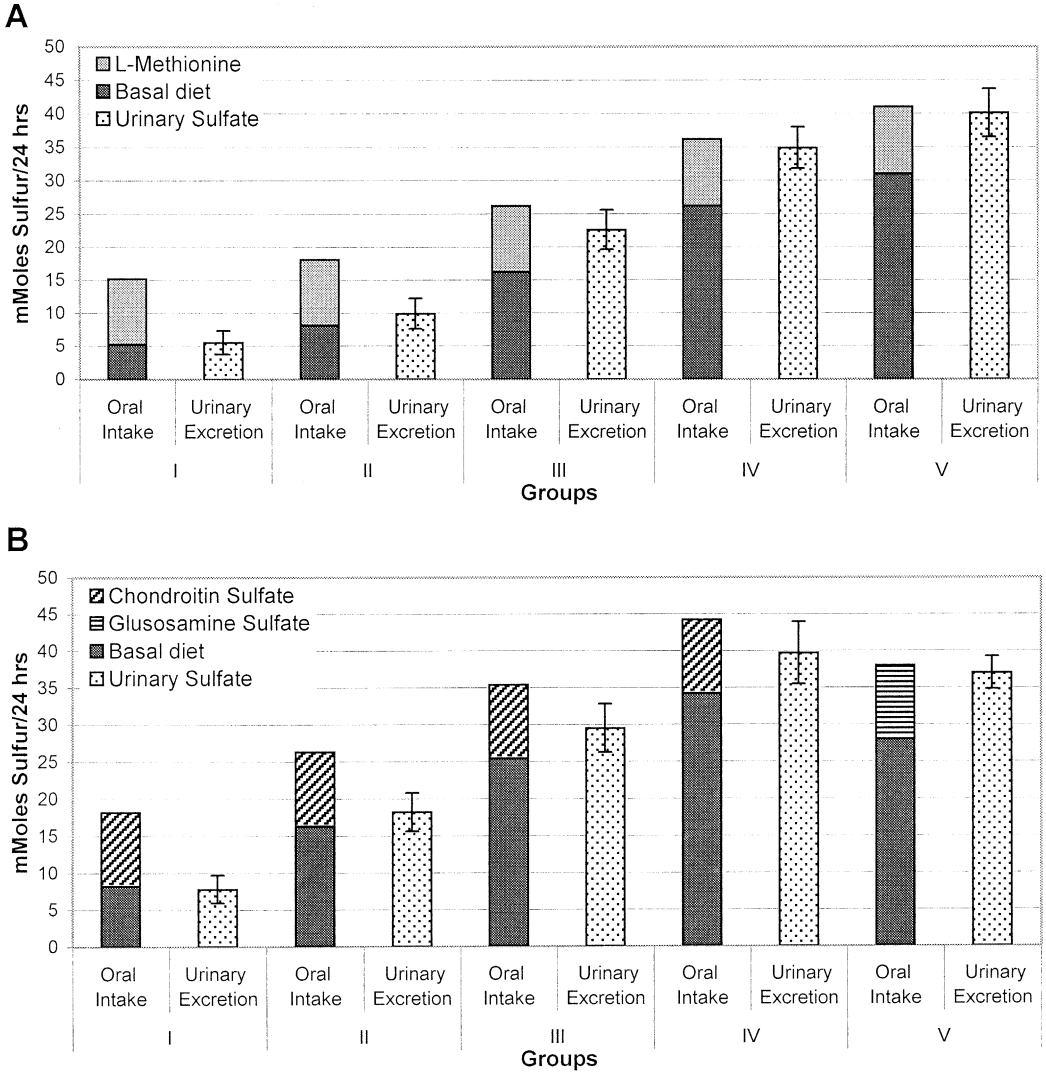

Fig. 1. Intake of SAA as part of the basic diet (dark bar) superimposed by the 10 mmoles supplement administered separately as a single dose on the morning of the experiment. Total height of the bar represents the total intake of SAA in millimoles. Urinary output of free inorganic sulfate included the standard error for four to five separate determinations. (A) L-Methionine supplement. (B) Chondroitin sulfate supplement (groups I–IV) and glucosamine sulfate supplement (group V).

the actual test. Protein levels were incremented by the addition of low-fat tuna, which is mostly protein (94% of dry weight is protein), to the basal diet.

Figure 1A summarizes sulfur balance studies which include L-methionine supplements. The dietary SAA intake of group II, 1.1 g or 8 mmoles, before including the supplement, is consistent with the RDA of the National Academy of Sciences (1989). Protein levels in groups IV and V, on the other hand, are more consistent with the higher requirements of older individuals measured in a VA Hospital setting (2.8–3.0 g or approximately 26 mmoles)[7], a value also reached by group III after including the supplement. It is apparent that only those individuals consuming the higher levels of dietary SAA tended to quantitatively excrete the methionine supplement as urinary sulfate. Lower consumption of protein was accompanied by sulfate retention, which we suspect as part of a sparing mechanism associated with inadequate intake of SAA. The small percent of ester sulfate excreted was disregarded, as none of these individuals were on medications.

A similar study, using chondroitin sulfate as a dietary source of sulfur, produced equivalent results [Fig. 1(B)]. Only at the higher levels of dietary protein is the chondroitin sulfate supplement excreted in the urine as inorganic sulfate. Inorganic sulfate derived from chondroitin sulfate is never quantitatively excreted in this group, probably because it is only partially degraded by the intestinal flora to inorganic sulfate, a pre-requisite for absorption. Studies performed over 70 years ago by Neuberger and Hoffman and later by Dohlman[8] showed that bacterial sulfatases were necessary to release the sulfate moiety of chondroitin sulfate and that rats receiving sulfa and antibiotics, which destroyed their intestinal flora, were not able to absorb chondroitin sulfate given orally. All glucosamine sulfate is essentially excreted in the urine as inorganic sulfate (group V). This observation confirms recent findings showing that sulfate concentrations in serum and synovial fluid increased significantly in individuals 3 h after receiving glucosamine sulfate[9]. It is important to note that this response was reversed by concomitant acetaminophen

administration. A lesser and non-significant increment was noted 3 h after 3.5 mmoles of anhydrous sodium sulfate was administered. Our experience shows that if a similar amount of inorganic sulfate is administered during the consumption of a diet rich in SAA, it will be excreted in a quantitative fashion over a 24 hour period. If on the other hand protein levels are inadequate sulfate will be retained. Although these findings strongly support the idea that the RDA for SAA are underestimated by using N-balance as a criteria, the significantly higher values that we observed could slightly overestimate requirements. Subjects were placed on marginal protein diets for up to 96 h prior to the initiation of the study, which could of depleted sulfur storages, such as glutathione, and enhanced sulfur retention.

It should be pointed out, for purpose of comparison, that the dietary SAA intake in a normal population ranges between 1.8 and 6.0 g of SAA/day (14–45 mmoles). A preliminary evaluation that we performed on 34 individuals fell within these values. The lower ranges, in the vicinity of 2.6 g/day, was achieved by vegetarians and individuals who restrict their animal protein consumption to small portions of chicken or fish and consume little or no red meats, quite fashionable nowadays.

Our metabolic data extends the suggestions that sulfate may mediate the therapeutic effects of glucosamine sulfate[9] to other sulfur containing compounds. It also suggests that the requirements for SAA should be reevaluated, not necessarily in the context of protein synthesis using the classical nitrogen balance approach, for which the current estimates will most likely hold, but for their ability to provide sufficient sulfur for the synthesis of proteoglycans and other important S-containing metabolic intermediates (coenzyme A, S-adenosyl methionine, glutathione, etc.), all of which play an important role in cell, and in particular, chondrocyte metabolism. Recent findings from the Framingham osteoporosis study[10] further support this line of thought, since individuals consuming a low protein intake presented with significantly less bone at femoral and spine sites.

It should also be noted that sulfate depletion inhibits GAG synthesis by rat and human articular cartilage, human articular cartilage being particularly susceptible[11]. The extracellular sulfate pool in humans is amongst the smallest of all species investigated[12], one reason probably being that the precursor-free sulfhydro groups are cytotoxic. This, therefore, requires a rather sustained supply of dietary sulfur. In summary, our findings offer a plausible explanation for the mode of action of chondroprotective compounds. By serving as a source of inorganic sulfur, they may be compensating for a sub-optimal or marginal intake of SAA. These studies suggest the possibility that increasing the intake of dietary protein of high biological value, or the regular consumption throughout the day of certain mineral waters, some of which contain around 0.5 g/l of inorganic sulfate, may prove to have effects similar to those derived from the administration of the sulfur containing compounds in question. It is recommended that the dietary intake of sulfur be monitored in studies designed to evaluate the efficacy of chondroprotective compounds, as it is possible that the individuals who benefit the most from them may be those with marginal protein intake.

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